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Chronic lymphocytic leukemia and Chronic Lymphoproliferative Disorders

XPO1 MUTATIONS DRIVE BCR HYPERACTIVATION AND EARLY DISEASE PROGRESSION IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Introduction: .*XPO1* encodes exportin-1, a key shuttle protein regulating nuclear–cytoplasmic transport. The recurrent hotspot mutation E571K has been linked to early disease progression in chronic lymphocytic leukemia (CLL). ATAC- and RNA-seq analyses revealed that *XPO1*-mutated CLL displays a distinct chromatin landscape with increased accessibility at transcription factor binding sites downstream B-cell receptor (BCR) and upregulation of *MIR155HG*. We hypothesize that mutant XPO1 enhances nuclear export of DUSP1, a nuclear phosphatase inhibiting MAPKs, leading to *MIR155HG* overexpression, increased miR-155 and reduced SHIP1, with consequent BCR amplification. This mechanism may be linked to the aggressive clinical behavior and shorter time to first treatment (TTFT) observed in *XPO1*-mutated CLL, although the precise molecular mechanisms have not been fully understood.

Methods: To dissect the downstream effects of the E571K, HG3 CLL cell line was engineered using CRISPR-Cas9. A control was generated in parallel. Clones were validated by next generation sequencing and analyzed by flow cytometry for immunophenotype, phosphoflow and calcium flux upon anti-IgM/IgD stimulation. XPO1–target protein colocalization was assessed by ImageStream and Western blot.

Results: HG3 CLL cell line was engineered though CRISPR-Cas9 to introduce E571K mutation together with two additional silent mutations disrupting the Cas9 recognition site, preventing further cleavage after repair. In parallel, a control cell line harboring only the two silent mutations was generated. All CRISPR-edited clones retained a CLL-like immunophenotype with lambda light chain restriction and surface IgM/IgD positivity. Both the XPO1^{E571K} and control clones carried homozygous del13q and wild-type *TP53*. To evaluate BCR reactiveness, calcium flux was measured. The E571K clone displayed stronger calcium flux upon anti-IgM stimulation ($p=0.009$) compared to the control (Figure 1A) and a similar trend was observed upon anti-IgD stimulation. This is possibly explained by higher phosphorylated levels of p-SYK ($p<0.0001$), p-BTK ($p<0.0001$), p-AKT ($p=0.009$), p-ERK ($p=0.0001$) compared to the control, suggesting a more active BCR signaling (Figure 1B-E). Furthermore, XPO1^{E571K} cells showed increased cytoplasmic colocalization of DUSP1 with XPO1, consistent with enhanced nuclear export. In addition, under serum withdrawal, *XPO1*-mutated cells showed reduced cell death at 72 hours compared to control, indicating increased survival under stress conditions.

Conclusions: Preliminary *in vitro* investigations utilizing *XPO1*-mutant engineered HG3 CLL cell lines demonstrate enhanced BCR signaling activity, corroborating prior transcriptomic (RNA-seq) and chromatin accessibility (ATAC-seq) analyses. Ongoing studies are focused on delineating the specific XPO1 cargo proteins that mediate this enhanced BCR signaling phenotype.

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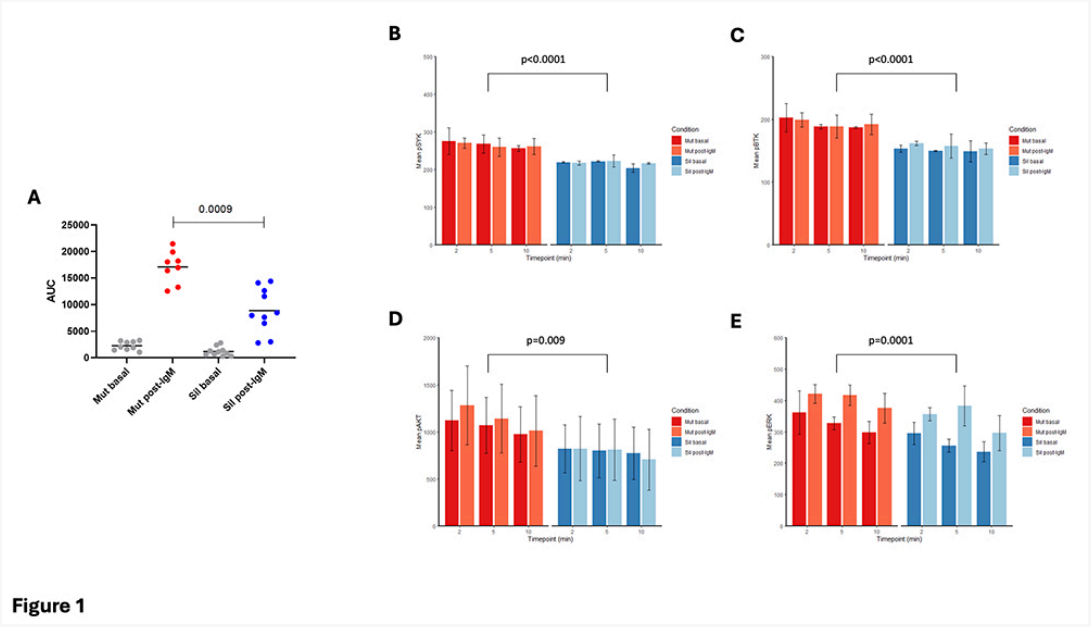

Figure 1